Course on Integrated Waste Management for a Smart City
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Module-03 Lecture-13
Chemical Analysis Procedure

Okay. Welcome back. So we will get started from where we left yesterday. We have been talking about I mean in the previous module, we have been talking about the like QA/QC, the quality assurance, quality control, the data quality of any whenever we try to do data collection. And the focus of discussion has been on waste management but as I said it is relevant for other aspect of environmental engineering as well or in general, even in chemistry or other wherever you use some analytical technique.

So yesterday if you remember, so in the last module if you remember we were looking at the QA/QC and then the next like blank, spikes, replicates. So one of the other important stuff here is to look at the method detection limit. So what is method detection limit, we will talk about, we will kind of start talking about that. And I will do a math to tell you how it is calculated. And that is another big thing.

Whenever you look at any environmental report where the data has been collected, it has been analyzed and it has been put in form of a table, we see that people reporting, many times people even reporting zero. That is actually wrong. You cannot report zero because we cannot measure zero. So since we cannot measure zero, we cannot report zero. Because any machine that you have, how sophisticated it may be, it can measure up to a certain concentration.

Below that concentration, the number that you are getting is not correct. It is, the machine will give you a number. That is where you as a master student, PhD student or a researcher or a faculty member or whoever is or a technician, whenever you are using analytical machine, it is many times I get the answer from my students saying that this is what I got from the machine. So it is like a things written in stone. That is not correct because the machine will give you a number but you have to make sense out of that number.

So you cannot report anything as zero because you cannot measure zero. So what the minimum concentration that you can report and that is the minimum concentration is known as the method detection limit. So for example, if you are using spectrophotometer and for to analyze a certain

parameter, so spectrophotometer can measure up to say 1 ppm or 0.5 ppm or 0.1 ppm or say 10 ppb. So there is a limit to which it can measure. Below that, the machine, it does not have the capability to identify that concentration because it is too low for that machine. So that concentration again, I am just trying to explain in different way. So that concentration, we will call method detection limit.

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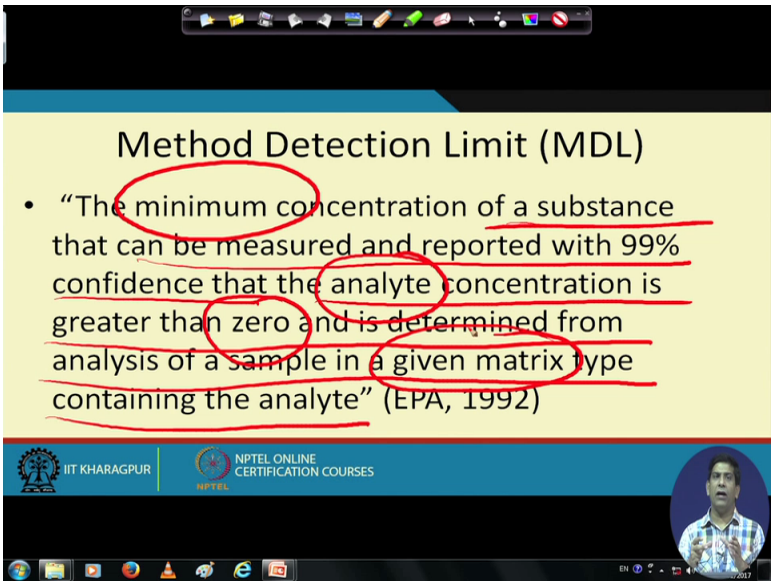

So if you look at this particular slide, this is a regulatory definition. That is why it is a big text. Do not, whatever I have been trying to explain you in last minute or two is what has been given over here. It is again, it has been directly copied from the text from one of the like regulatory document. So that is why is the minimum concentration. So if you look at here, it is what it is telling you, whatever I was trying to explain to you, it is the minimum concentration, it is the minimum that of a substance or we can call it an analyte. That can be measured and reported with 99 percent confidence.

So if you again your statistics, if you remember from your statistics class, if you have not taken one, I encourage you to take one. Because for any in, whenever you do the data collection for and try to do the data analysis, statistics plays a big role. You should have a fairly good understanding of the basic statistics, all those T-test, G-test, ANOVA, Chi-square and your regression analysis, looking at the confidence limit, confidence interval. So those things are,

those are very basic in terms of the statistics and then you should be aware of that. We cannot cover that material in this class but that those materials are useful.

So if you really want to do well in this course, I would encourage you to go and read about it. If you have trouble finding source for that, send me, send us a question on discussion board. We will be happy to provide you link to some good resource on statistics, especially on environmental statistics but it is your responsibility to learn that. We will not going to cover that in this particular course. And most of you must have taken a statistics course by this time.

So it is a minimum concentration of any substance that can be measured and reported with 99 percent confidence. So it is we use 99 percent, 95 percent, 90 percent. So with the 99 percent confidence and what is that? That is the analyte concentration is greater than zero and is determined from the analysis of a sample in a given matrix containing that analyte. So I will try to explain that one by one. Analyte means there are parameter that we are trying to measure, whether it is a arsenic, lead, the cadmium, whatever you are looking for or any organic chemical that you are looking for. So that is the analyte that you are trying to measure.

The concentration of course is the amount that is present. Concentration, we usually we express them as mass per volume or mass per mass. So mass per volume will be like milligrams per liter, mass per mass will be milligrams per kilogram. So that is the analyte concentration and the value is greater than zero. That is we can say with the 99 percent confidence that the value is greater than zero. And we determine that number in a given matrix. Now what is the meaning of given matrix?

In the previous module also I used a term matrix spike. So given matrix means what kind of sample you have. Do you have a clear water sample? Do you have a surface water sample or a waste water sample or a landfill leachate sample or it is a sandy soil or clay soil or a organic soil? So depending on the type of matrix, those are your matrix like where our analyte is present. So in that particular matrix, so once, because depending on the type of matrix, your analytical capability of the machine also keeps on changing.

So what I am, again I will try to just to illustrate like just to elaborate on that. If you are using DI water or Nanopure water, DI water, Nanopure water other than the chemical of interest that you have, there might be some other chemicals present there. But overall, it will not be of a nasty

kind of material. It is not a nasty material, there is not lot of other things in there. It is a clean water, so it is analysis becomes little bit easier.

But when we go to landfill leachate or a waste water, industrial waste water, there are lot of things there. It, there is a lot of organic matter present, there could be sulfide. So there will be lot of interferences of all the different parameters that is present in that matrix or in that media. So their analytical capability may not be the same as what it was for the DI water. So that is why the matrix is very important. That is the reason we use the matrix spike that I explained in the previous video.

So to know whether in that particular matrix, we are getting the good response, so similarly here when you are trying to look at the detection limit, your detection limit may change depending on the type of matrix that you are using. So it is very, very important to know because depending on the different types of matrix, the complexity of the problem changes because they are lot of different things will be present and they will try to interfere. So that is called interference. You will have interference problem. So those, so they may be competing for same kind of reaction. So there is lot of things can happen which, it is difficult to explain in this particular course.

This is a, that is basically analytical, analysis course and there might be some courses like that either on NPTEL platform or these days you can have VDX and other platforms there too. So you can look at that. There are some good books on that as well. But for just for a normal understanding, you should try to understand that based on the matrix, our analytical capability changes for the same machine. So that is why when we try to go for this detection limit, we have to know what matrix for which it was determined.

So again what is the detection limit? Detection limit is the minimum concentration which can be reported using a particular analytical instrument with 99 percent confidence interval that the number is greater than zero and the number is like it is we are 99 percent confident that the number is correct. So that is, now we get it? I will not go in very much basics of that but just to kind of show you like how like what is the method it is being used to and to get this method detection limit.

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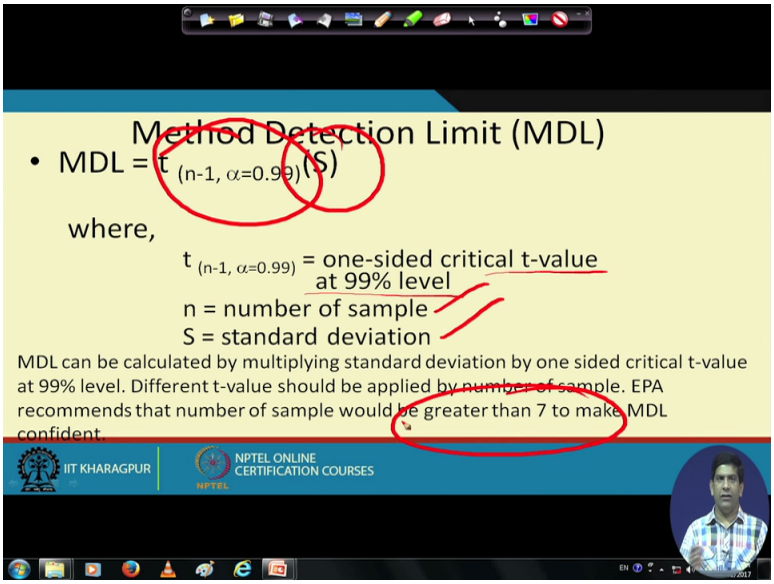

So in terms of the method detection limit, what we do is there is we use this formula which is MDL which is method detection limit. It is your t, t from your t table. Let us go back here. So your t is your t table and then S is the standard deviation. Now what is this t? How we got the t? How we got the S? We will talk about that in a minute. Where t is your one-sided critical t-value at 99 percent level, n is the number of sample, S is the standard deviation.

So what we do, we take, we multiply the standard deviation by one-sided critical t numbers. Different t value should be applied based on the number of sample. EPA recommends that minimum or any regulatory agency requires you to have minimum at least 7 samples. So what do we mean by these minimum 7 samples? I will explain it in the next slide using an example, so that will make you clear what we are trying to say here.

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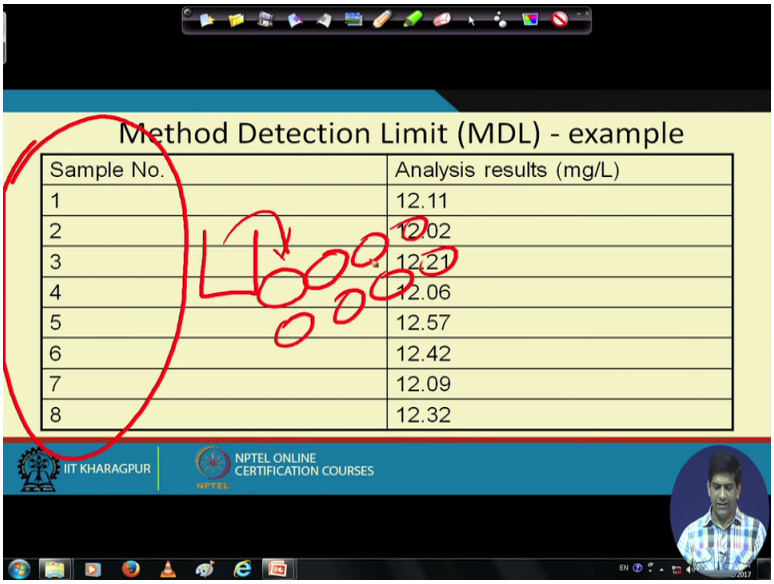

Say this is an example. So you want to analyze, you are planning to analyze for one particular parameter. Let us for our discussion sake, let us take this data, is for lead. So here we had a sample. We did, this is we did different replicates of the same sample. So we had 8 replicates. It is a same sample. So basically it is the same sample and we have taken out and did 8 of that. 1, 2, 3, 4, 5, 6, 7, 8; 8 of the same sample we have done. So they are like 8 replicates. So 8 times it was analyzed, let us clean this up.

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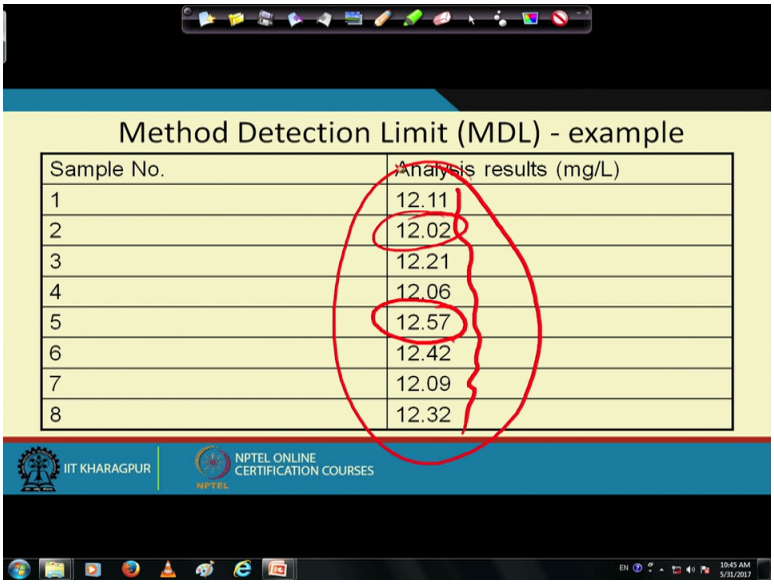

So 8 times it was analyzed and these are the numbers we got. So the numbers are like 12.11, 12.02. The minimum one we have is 12.02 and the maximum we got is 12.57. And then it varies between 12.02 to 12.57, so there is not a lot of variability. So if you have this kind of variability which is almost like plus+ minus-, even less than 10 percent, so that is really a very good analysis.

So your instruments are working really well and you are a good, your lab practice is good. Like your hands on the lab activity is pretty good because you do not see that much of variability. So this is a good dataset. And these are all, all these I will show you several datasets in this set of slides in this video and they are all made up just for illustrative purpose. They are not real data. Just to explain things here.

So but you take analyte. If you want to analyze say lead on ICP or you want to analyze arsenic on ICP or arsenic on graphite furnace, GFAA or you are doing arsenic on flame, there are different things, there are different instruments we can use. You can do certain things on GC. So for any analysis that you are doing, you will take a sample and you will do 8 replicates of that. Minimum is required is 7. So you do 8 replicates and we got these numbers.

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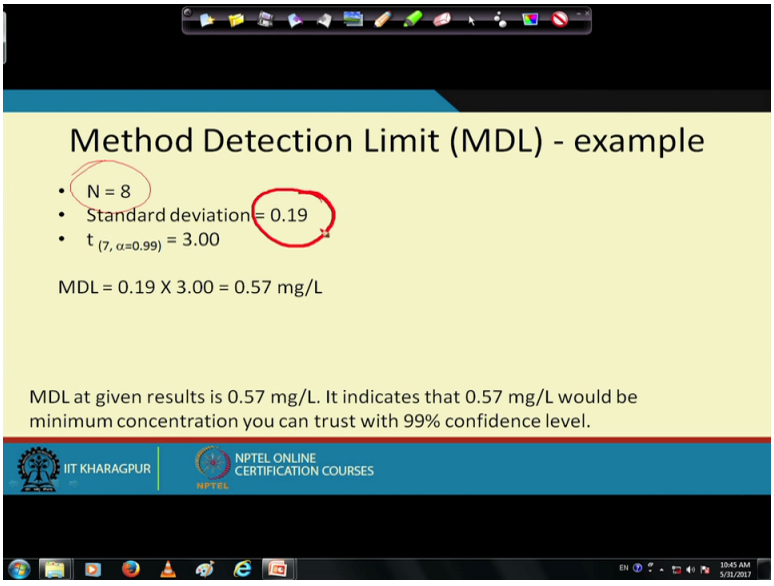

So now what we do with these numbers? So after these 8 replicates that we got, what we will do is we will use that equation. That is, that was we were looking at in the previous slide where now the n is equal to 8. Our number of sample, n was 8. We can calculate the standard deviation.

Standard deviation for this sample if you do, I encourage you to test, check out these numbers. So do not just trust me, I may also make some mistake. So you trust, you check those numbers. Your data has been given in the previous slide, so and you will have the PDF of this whole lecture material with you as a reading material too.

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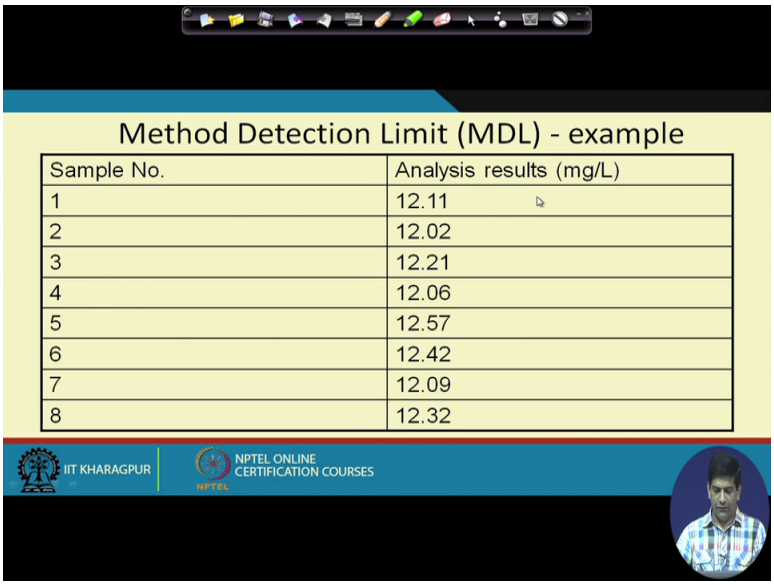

And so standard deviation is 0.19 of the dataset that we just looked at in the previous table which was this table. So if you look at this table, the data, the standard deviation is 0.19.

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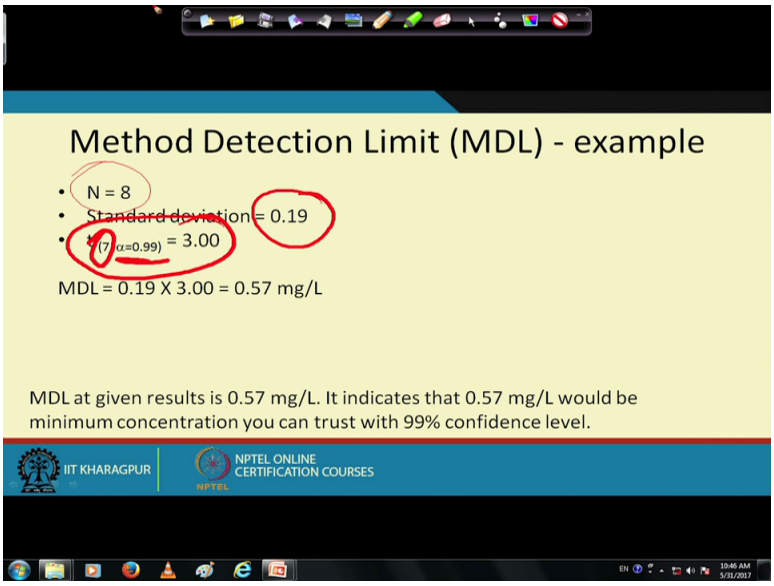

And if you go to t table from any statistics textbook, why it is 7? Because remember that degree of freedom is n minus- 1. n is 8, so n minus- 1 is 7. So that is why we have this 7 and excuse me, so this is, that is why we have this 7. And then we set the alpha at 99 percent confidence, so that is why alpha is 0.99. So if you go and look at a t table from any statistics book, the value is 3.

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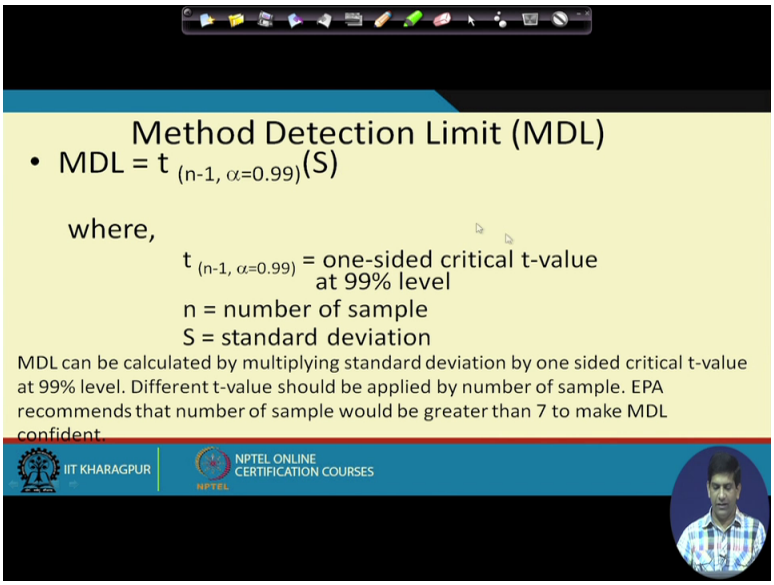

So what is our from that, if you remember the formula that we used and the formula that I showed you in the previous, t n minus- 1 alpha. So this value is, we got it from the t table, times the standard deviation.

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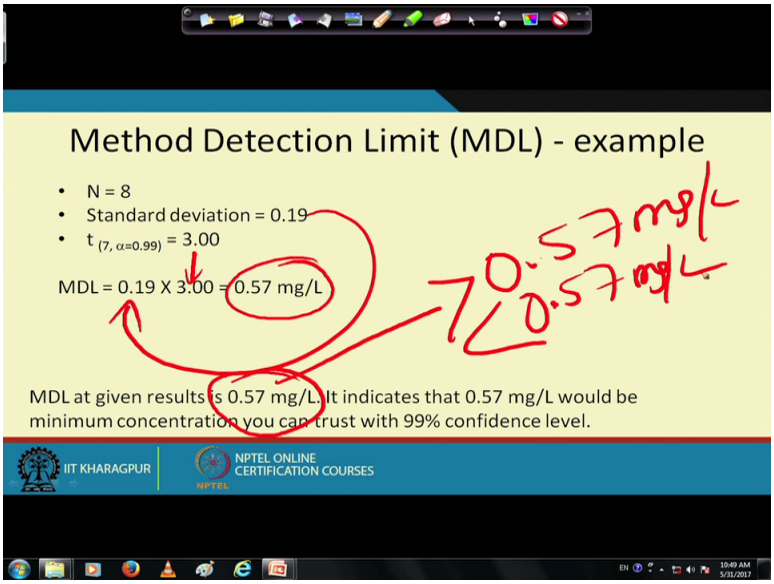

So if you come back here, so this t value is 3. We got 3 from here. So sorry, so it is t value is 3, so we got it there. Standard deviation is 0.19, that is what we got it here, multiplied that and we got the value of 0.57. So that is MDL for this particular analyte. In this particular matrix is 0.57 milligrams per liter. So what does that really mean? It means that it is 0.57 milligram per liter would be the minimum concentration you can trust with 99 percent confidence level.

So what that again, what is to elaborate that? So if you have, if you do this analysis for unknown sample, say for our discussion sake, we said okay, this is for the lead, we are analyzing for lead from some water sample or soil sample and we got this concentration. So it is for lead and for that particular water sample or it could be e-waste leachate, whatever. So it is when we do a unknown sample, the concentration if it is above 0.57, if the number is above 0.57, so if it is greater than 0.57, we can rely on that concentration. If it is something less than 0.57, we cannot say for sure that the concentration is correct because the machine cannot measure something with 99 confidence level of any concentration less than 0.57.

How we know that? Because we did that procedure as prescribed by like based on our statistical, statistics finds. There is actually lot of books out there on environmental statistics. That US EPA and several regulatory agencies, if you go on Google and try to look for environmental statistics handbook, you will find several of those handbooks which gives this, how to do this. And to get into that background, that will be like little bit of, lot of statistics involved, so we will refrain

from doing that. If you are interested, you are more than welcome to read about it and ask questions on that. But it is, so anything greater than 0.57, we are sure, we are 99 percent confident that the number is correct. Anything less than 0.57, we are not confident.

So when you do this analysis of unknown sample, you may get a value of say 0.4 or 0.3 milligram per liter. But that number does not make any, although the machine will give you a number, but as a researcher you have to decide and you need to say that no, this is below detection, so I cannot trust this number. So number cannot be, it may not be true because it is below the detection limit. That is, it cannot comprehend that number. The machine does not have the capability to comprehend that number. And that is very important.

And again that is another thing like I have been reading so many, as external examiner, I get lot of theses, PhD theses coming to me from different Indian institutions as well. And I have done that while I was abroad as external examiner and now I am doing as Indian examiner. So that is one thing I see that, many times the detection limit is not even reported. Students do not even understand the concept of detection limit.

So these are really very, very important concept, especially if you want to collect good data. And good data is kind of the raw material for any good design. If we cannot have good data, our design will have problems. And we have seen that happening especially in the solid waste field over last 10, 15 years where we have been designing plans after plans and those plans are not working.

And one of the, there are other reasons out there but one of the major reason for that is having bad data to start with. And the data was, the data that was used to design that plan was not correct. And there is different reasons associated with that as well which we talked about. So anything less than 0.57, what you will do with that? We will talk about that in a minute as well. How you handle something which is below detection limit? Because that is also is some sort of information out there. So but this is very, very important concept. If you, I will encourage you to kind of look at this part of the video again and again to make sure you understand. If you do not, feel free to ask question through the discussion board. No problem there.

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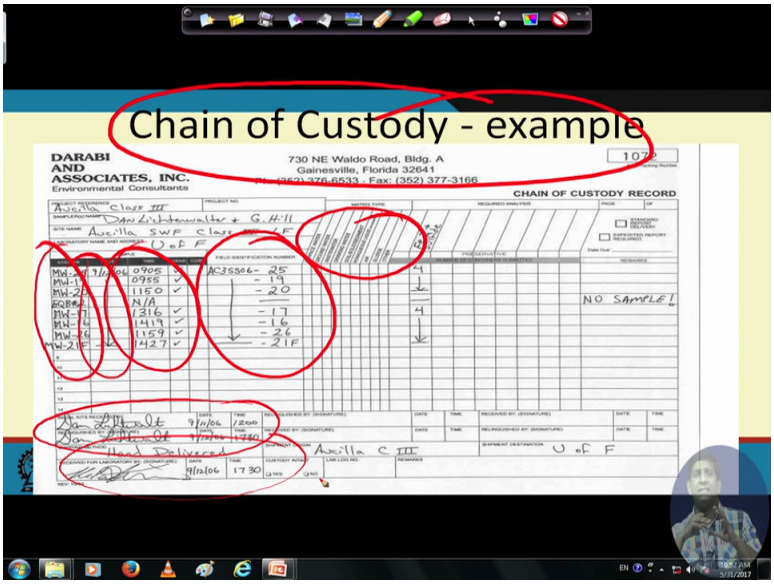

Some other things that we do in terms of the QA/QC, we need to track the sample. The tracking of the sample is done using some sort of a document called Chain of Custody. We do not, like we will like to talk about that but these other things that we talked about is more important than this. But this is also important component where basically you need to track the sample. When the sample was collected, this is like a sample ID, when it was collected, at what time it was collected, what is any field identification number, where it was got collected, what are the different parameters we want to analyze it for.

And then like who, then there is a signature kind of who collected it, what time and then once you give it to a lab whether it was a hand delivered, sent through a FedEx or UPS or even some sort of courier or and then when it was received, who has received it, so there are multiple copies of it. And once you get a, you get the analysis report, you get this Chain of Custody, copy of this Chain of Custody with that as well so that you can track how the sample was collected, who has collected.

So that is, the reason for that if there is any problem with the sample, if there is any problem with the data and you are trying to track where the problem is, how problem got into, you can talk to the people who collected the sample, get their some of this personal observation which did not get reported, which did not get documented, so you can do that doing that. So this Chain of Custody is very, very important from a legal perspective as well.

So when you go for, say something happens and it goes into a legal issue, these days in our country with so much of environmental litigation going on, NGT and other things are involved, so we have to be careful. We need to follow a good quality assurance, quality control of any projects that we are going to do. And it is a good idea to do that even for your masters theses and PhD theses as much as possible so that you establish some good practices in the lab.

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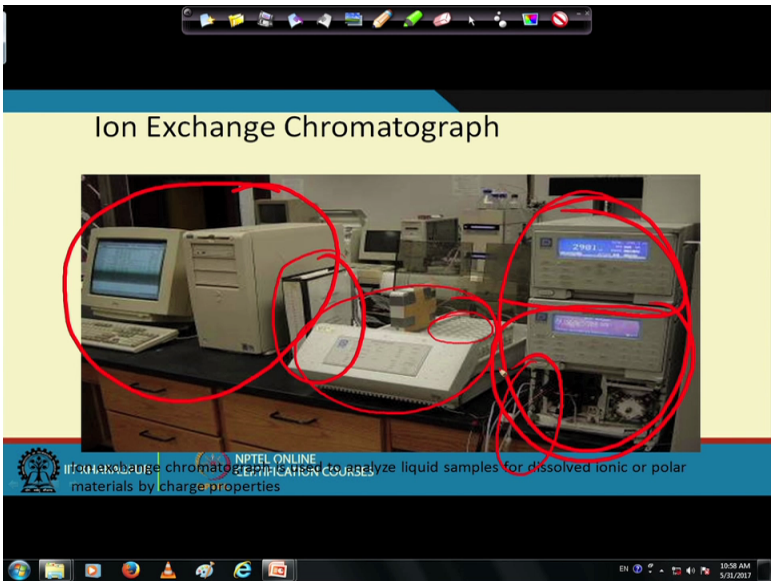

So let us look at some of the instruments and then we will talk little bit more about this, our this method detection limit part where how to handle something which is below method detection limit, we will talk about it. Let us look at quickly some of the different instruments which we use. So one of the instrument which you may have seen and if you are being to any environmental lab is Ion Exchange Chromatograph.

Now what is that Ion Exchange Chromatograph? What is, the chromatograph, you hear this chromatograph used a lot in like we have IC, we have GC, we have HPLC, we have LC. So LC is the liquid chromatograph, IC is the ion chromatograph, GC is the gas chromatograph. So you always there is a chromatograph. So what is that? What is the meaning of this chromatograph? Chromatograph essentially what it does, it say if you have a sample, you are pumping this, this is your column and you are pumping the sample through. And this column has some material in there, and that material helps separate the different fractions present in the sample.

So for example, if you had a sample which has some sulfide, has nitrate, nitrite, bromide, fluoride, just if you are talking about anions and sulfate for and then once the sample passes through this, since the affinity of these different anions are different to the packing material present in this particular column, things will get separated out. And based on in a very simple way if you think about that, you can think that this particular column just we put some material in which is called the packing material.

So let us assume that packing material like having lots of chairs in there. So there are lots of chairs. So once you pass through this and all those chairs are occupied by this, what we, first we pass through what is known as (())(21:25) material. (())(21:26) material which has the highest affinity for that particular chair, it will go and it will occupy all those positions. Now when we are passing this through the sample, if the sulfate has more affinity than this particular (())(21:40) material that we said earlier, sulfate will kick out that (())(21:44) material and occupy that seat.

So that is how it is done. And then so it is based on that, it is those things gets occupied first and then you pass through a substance which is higher affinity than all these anions and then one by one these anions will be taken out. So more the affinity, that particular anion will come out later. And that concept is called retention time. That concept is called the retention time.

Later, so the material first of all, gets separated into different components and then based on their affinity to the packing material and how hard they can fight with that (())(22:24) material, it is when the different times it will come out. So this was explanation more in a general way. So do not give this, do not write like this in your exam because I was just trying to explain you in a non-technical way. But in the exam, you have to actually write in a technical way where you kind of talk about, in this solid waste class, we will not ask you those kind of questions but if you are doing analytical course, environmental analysis course or something like that where these kind of questions may be asked.

But again it is a one way of analyzing different anions and we can also do cations. But with the advent of ICP now or even AA, most of the cations are actually done using ICP or AA. And if you do not have those, many places they use spectrophotometer. Spectrophotometer is the most common instrument you see in most of the lab. Even some of these anions can be done using a spectrophotometer as well. So there are different ways of doing the same analysis.

So just you need to make sure you mention that clearly which analytical instrument you use, what was the detection limit of that analytical instrument and then you report that accordingly. So this ion exchange chromatograph, it is basically for anion. So here you can see there is a, as you can see over here, it has different components, so we are essentially looking at this. So there is of course, this is an older version. The newer version, looks much smaller, much compact. So as you can possibly see from the computer itself, this is older version.

So there is a computer, it is a computer controlled. All of these are computer controlled these days, so there will be a computer control. Here you see the lab manual which has all this QA/QC procedure and how to do all that standard operating procedure and all those things are there. This part is our autosampler, so you can prepare several samples and this here you can see several holes. And this part, you can put all your different samples there. And those different samples can be analyzed at one go.

You set up your instrument and it will be analyzing if for 5 hours, 6 hours. You can come back and watch on it from time to time. The newer version, even you can watch on it using your smartphone. So there is app where you can look at the how this machine is working. And you can even set down the machine using that app but you cannot restart the machine. So you have to go back to the lab to do that. But there are lot of new things are happening.

And this part is the detector part. So this is the detector part and this is the main machine part. The top part is the detector and the bottom part is the column. So actually this top part is the detector part and this bottom part is where we have this column. The columns are over here, the picture is not that very clear. But there that is where the columns are. So what the column does, it separates different fractions and the detector based on different anions have different level of conductivity. So it works on the basis of, sorry, anions have conductivity. So based on the conductivity numbers, you can predict what will be the concentration.

So before you go for that unknown sample, you will again do the calibration curve and you will know the retention time for different anions coming out. So based on that, you can do the analysis part of those. So that is kind of the basic principle of most of this machine. So ion chromatograph used for anions, you can do it for anions, also can be done for cations as well. So that is one machine that we use quite a lot.

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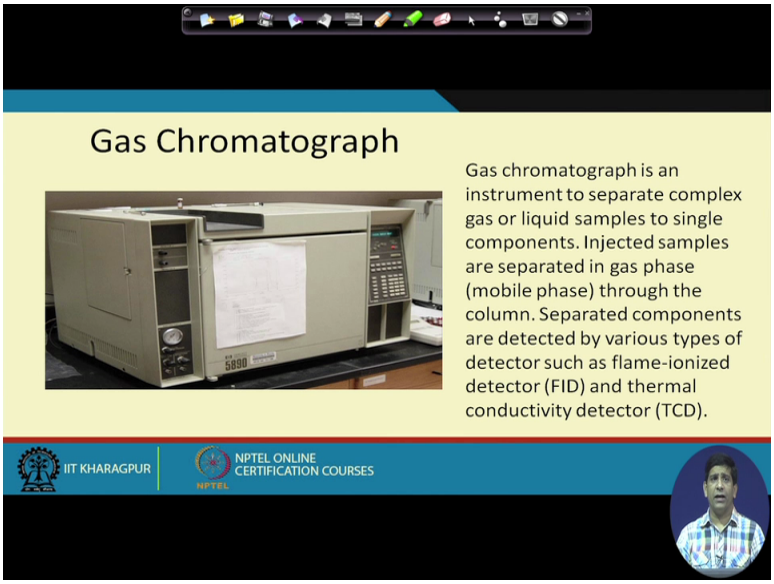

Then other is the gas chromatograph, gas chromatograph is an instrument to separate again complex gas or liquid to single component. So again injected sample are separated in gas phase and then separated components will be detected by various types of detector. There is a flame FID, which is a flame-ionized detector or thermal conductivity detector. So based on what kind of analytes you are looking for, whether you are looking for methane, you are looking for some organic gas or any different organic compounds, you will use different types of like a detector out there. So this is GC is used.

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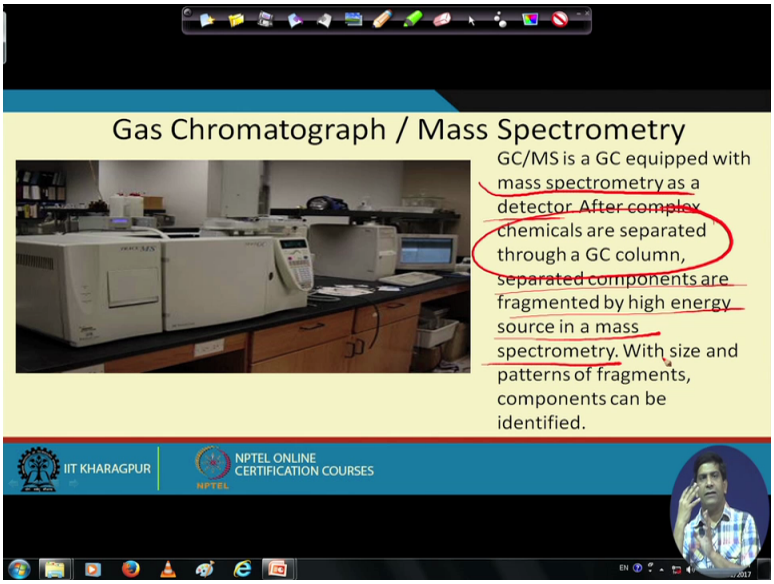

Then there is a GCMS. What is the difference between GC and GCMS? GCMS, in GC we try, we are actually trying to do the analysis of the sample for which we know what we are looking for. We are looking, say we are looking for methane or we are looking for benzene or toluene or xylene. So we know that we are looking for these compounds. In GCMS, if there is certain unknown in the sample, we are not even sure what kind of, what could be there. And that is, most of the time, it is true for environmental samples. So you had environmental contamination. You would want to find out what are the different compounds present there in this, from this environmental contamination.

So you take your sample, you pass it through GCMS. What the GCMS has, it has, it is a library. The library is present. So there are based on the library it has had, based on its mass and charge ratio. And they will give you a, so after it is equipped with a mass spectrometry and so it is has this mass spectrometry is there which is as a detector. So it is m by z ratio which is a mass by charge ratio. It is many times, that is used.

Once the complex chemicals are separated through the column, the separated components are, they pass through a high energy source in MS and with size and pattern of fragments. So based on that the library that it has, it is trying to match the compound, it is trying to see with it what it has in the library. So in the library we have kind of different properties of the different chemicals

already given into the computer, in the database of that particular instrument. So it tries to match it.

And whichever closest matches, then it will say that most likely it has this contamination is benzene or toluene or xylene or TCE or PCE or those kind of matter. So then we will go back and test for that particular compound and then using the standards of that particular compound and just to verify whether that is present or not. So it is little bit, it is a complex. It is, so to maintain GCMS, to maintain ICPMS, to maintain LCMS or HPLC, it requires sophistication there as well. We need some good quality hands-on trained people to run those instruments for us. It is not an easy instrument but it is not a difficult thing to do as like anything, nothing is impossible. So it can be done but you need, we need to have some diligent, diligence, due diligence there. So that is on GC or GCMS.

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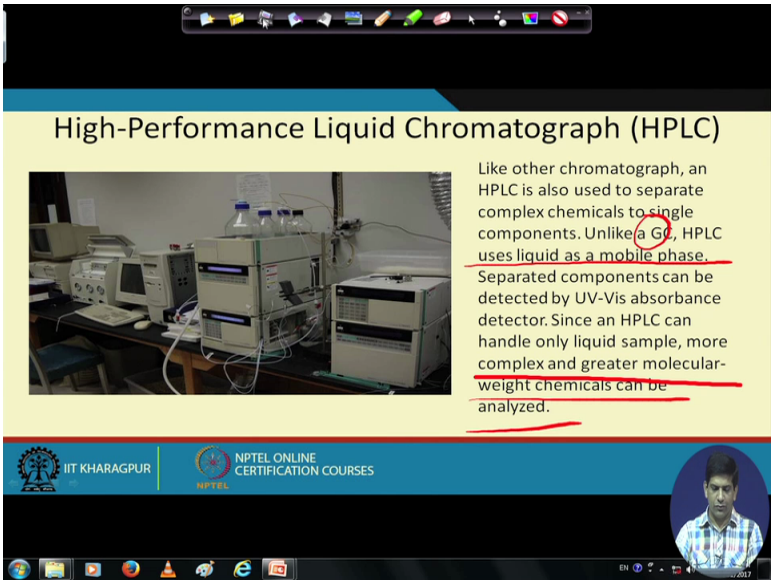

The next is HPLC, it is like other chromatograph, HPLC is also used. HPLC is usually used for more complex and greater molecular weight compounds. So usually for more complex and greater molecular weight compound can be analyzed for that. Here other than, unlike GC, it uses liquid as the mobile phase. And again we will not go into great detail about these different instruments. If you are interested, you can give me a call and we can talk about that. But if in particular instrument but I would encourage you to take instrument analysis course and I am pretty sure there should be one out there.

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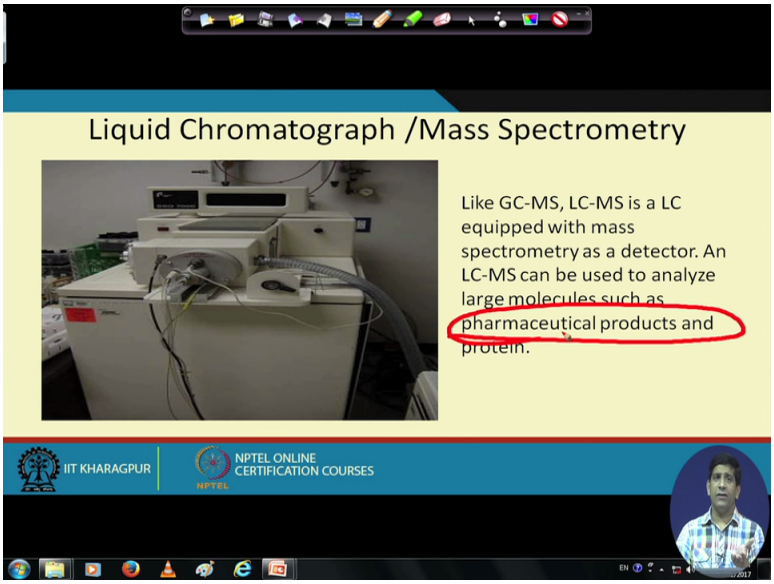

So then there is a LC. Like GCMS, there is LCMS which is used to do pharmaceutical. These days there is a lot of talk about pharmaceuticals product getting into our water bodies. So pharmaceuticals and personal care products, LCMS is used. Some of these instruments like LCMS is pretty expensive. It is we are talking about things in crores. So it is expensive instrument, so it does require lot of sophistication there as well.

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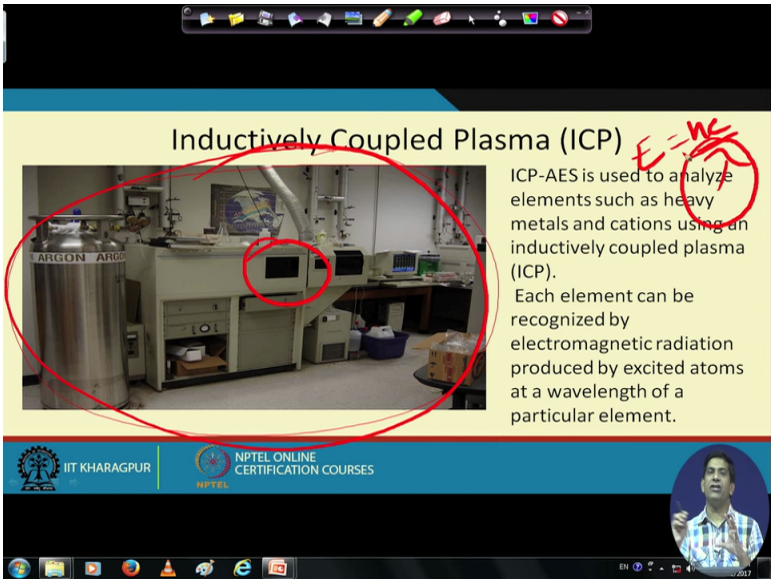

So this LCMS, then ICP which is used to do heavy metals. Each elements can be recognized, there is a, heavy metals are done for ICP-AES. These days again, this ICP-AES is a very, very

old machine. This is the machine that I used for my PhD, so I am kind of still using this picture but the newer machine actually is even less than half of the size. But I have emotional attachment with this picture. This was kind of the machine that I used for my PhD work at University of Florida. I have worked on this machine for nearly, after PhD even I worked there as research scientist. So nearly for five, six years this machine was my baby.

So it is, I was the person who was handling this machine day in and day out. So I have like a very strong attachment to this machine. This machine is still there. And it is, they have some new, newer machine there too. But this is, it is used for heavy metals like is used to analyze heavy metals and can be done for cations as well. And you use (IC), ICP essentially it works on atomic emission spectroscopy. So you take it, you excite all the things at a very high temperature. It works at around 5,000 to 10,000 degree centigrade. There is a torch which is right over here in this particular box. You cannot see it from that.

But since that box and things get pumped in the air and the sample gets pumped and when it gets burned to at 5,000 to 10,000 degree centigrade, it goes to excitation state. And if you remember from that, e is equal to hc upon lambda. That is if you, that will remind you of your like a modern physics from class 11, class 12 where h was the Planck's constant, c is the speed of the light and the lambda is one for a particular wavelength. And this lambda is unique to each and every element.

So for example, if you are trying to analyze for arsenic, arsenic has a specific lambda. Lead has a different lambda and cobalt or cadmium has a different lambda. So based on the, when you, when it gets into the excited state, if you remember from the Rutherford model of the atoms, so the electron has a tendency to come from higher elevated state to a normal state. And that is because it tries to stay in a normal state. So when it will try to come down, it will emit light.

And that light that will be emitted is, can be, is hc upon lambda and when the lambda is where it is unique to each particular element. So based on the intensity of the light for that particular wavelength, we correlate that to the concentration in the sample. So such a neat, is not it? It is such a beautiful concept. But I am really, anyone who come up with that idea, I do not know who but it is a great idea. So we are based on the, since the wavelength is unique to each and every element, there are some interferences there.

There are some interferences, sometimes it, you may get some false positive, false negative numbers and all those things and but when you work in the lab, you will understand that and you will appreciate that. But again based on, since the lambda is unique to each and every element, you can get the concentration of the sample based on the light that is coming out. So that is on ICP part which is one of the widely used sample for heavy metals.

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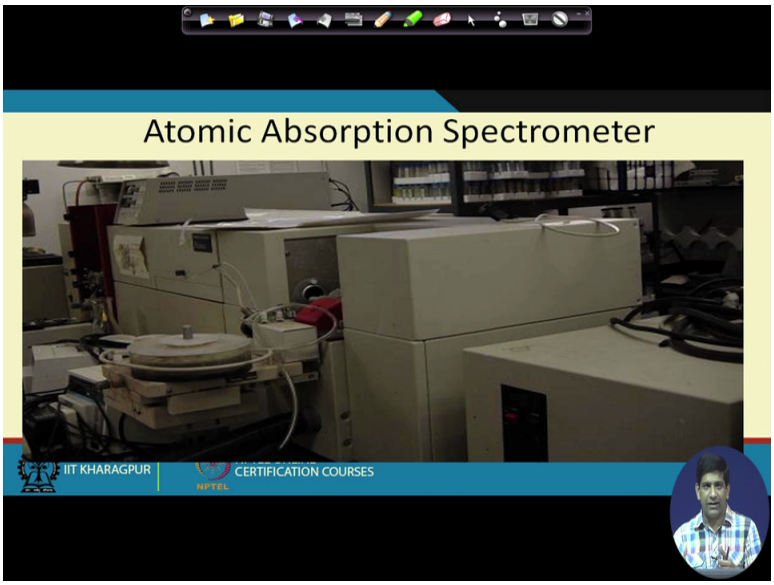

Atomic absorption again can be used for heavy metals too. The difference between atomic absorption and ICP is atomic absorption, you do it, it is based on absorption. ICP was on emission and graphite furnace you can do one sample at like one element at a time. ICP you can do 25, 26 elements at a time.

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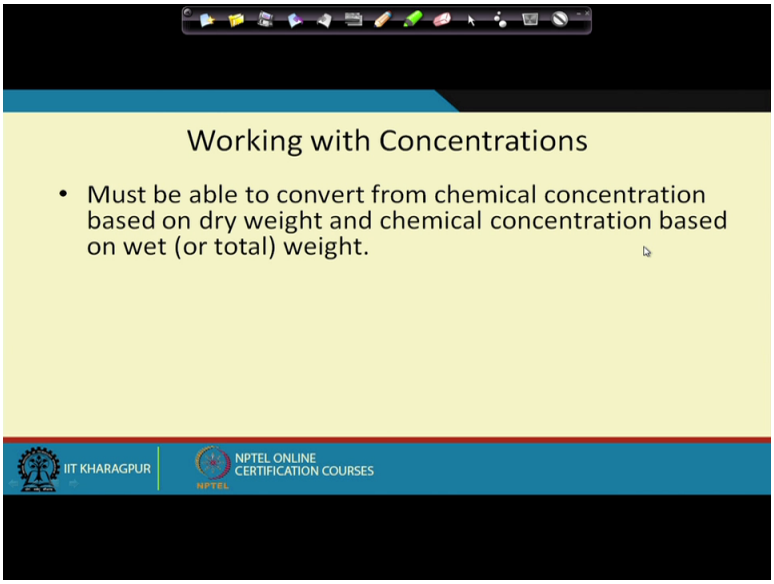

So once you have these done, then you have to work with the concentration. So once you have these analytical instruments, you have analyzed different parameters, you got the samples, you have used this analytical instrument that we talked about, you are getting the data. Now how to make sense out of that data? So in the next module, we will try to understand how to make sense out of this data and how to work with this data from a statistics point of view and other things. I will give you some examples and I will try to explain that.

So again in this particular video, what we tried to do, we tried to, looked at all the different instruments that are used for and this is not the complete list. It is just some example instruments, most common instruments that we used for analysis of environmental samples. And some of the issues associated with that or the method detection limit and how some, I tried to explain you very basics how these different instrument works but that was, it is not instrumental analysis course, so do not, I would encourage you to look at some instrument. If you are really interested to learn about instruments, look at some instrumental analysis book or course for that.

So in the next module, we will start looking at once we collect data from this instrument, how to make sense out of it. So let us, thank you again and again any question, discussion forum is there, and then I will again see you in the next module. Thank you.